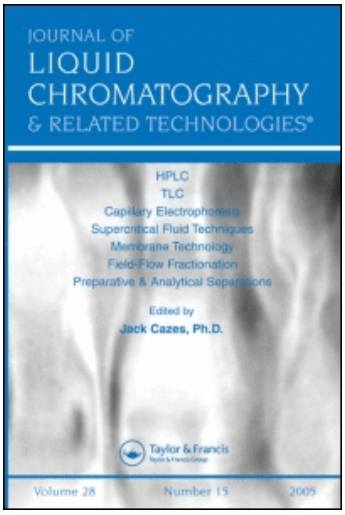

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Experimental Investigation of the Oscillatory Transenantiomerization of *L*-Tyrosine

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Abstract: In our earlier studies, we demonstrated an ability of selected enantiomeric profen drugs (e.g., *S*-(+)-ibuprofen, *S*-(+)-naproxen, and *S*-(+)- and *R*-(−)-flurbiprofen) and one amino acid (i.e., *L*-α-phenylalanine) to undergo oscillatory transenantiomerization when dissolved in simple, low molecular weight solvents (e.g., water, ethanol, dichloromethane, acetonitrile, etc.) and stored for a longer period of time at ambient temperature or in a refrigerator. Experimental evidence of this process originates from a number of analytical techniques, with thin layer chromatography (TLC) and polarimetry among the best performing ones. There are two common structural features of all these compounds, namely: (i) they are 2-arylpropionic acids (2-APAs), and (ii) their chirality center is located on the α-carbon atom of the respective molecules. It has also been established that the basic and the amphiprotic environment catalyzes the oscillatory transenantiomerization of the investigated compounds, while the acidic environment tends to hamper this process. Moreover, it has been established that all the aforementioned compounds can organize molecules present in the solution in such a manner as to produce the density anisotropy of the liquids considered. Model explanation of the oscillatory transenantiomerization of profens and *L*-α-phenylalanine was also developed as a starting point, adapting an earlier established oscillator known as Templator. The new model comprises two linked Templators. The quintessence of the Templator model adapted to the demands of the oscillatory transenantiomerization of profens and amino acids was based on an assumption

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that the H-bonded 2-APA dimer is a template, able to generate the new dimers having the same steric configuration of their respective monomeric units. From our earlier studies, it clearly comes out that in spite of common traits of the oscillatory transenantiomerization of the selected profens and *L*-α-phenylalanine, the dynamics of this process can significantly differ from one compound to another, due to their differentiated molecular structure and, hence, to the different electron density distribution. Thus, in this study, we investigated the ability of *L*-tyrosine (another 2-APA and the amino acid regarded as essential for the humans) to undergo oscillatory transenantiomerization. Solubility of *L*-tyrosine in the amphiprotic binary mixture (70% aqueous ethanol solution) widely used in our earlier studies proved too low to use it as a solvent in the present investigation. Instead, we traced the behavior of *L*-tyrosine when stored for over one week in the following mixed solvents: ethanol–1M NaOH (7:3, v/v) and ethanol-1M HCl (7:3, v/v). The results of our experiments clearly confirm the ability of *L*-tyrosine to undergo the oscillatory transenantiomerization, similar to that of the previously studied profens and L-α-phenylalanine, although the individual dynamics of the oscillatory transenantiomerization with this particular enantiomer is also evident and discussed. It is apparent that the model of the two linked Templators applies to *L*-tyrosine as well, as an adequate explanation of the mechanism of its oscillatory transenantiomerization.

Keywords: Keto-enol tautomerism, *L*-Tyrosine, Oscillatory transenantiomerization, Polarimetry, TLC

INTRODUCTION

In our earlier studies, we have reported on the phenomenon of the oscillatory transenantiomerization of selected profen drugs in aqueous, aqueous-organic, and purely organic solvents.[1–4] In another paper,[5] we presented the analogous phenomenon occurring with the dissolved samples of *L*-α-phenylalanine. It is noteworthy that profen drugs and *L*-α-phenylalanine can be ascribed to the same class of chiral 2-arylpropionic acids, with their chirality center located on the α-carbon atom of the respective molecules. This structural similarity seems an important precondition of oscillatory transenantiomerization, with the main feature of the steric conversion summarized with aid of the following equation:

$$(+)\text{-2-APA} \leftrightarrow \text{keto-enol tautomer} \leftrightarrow (-)\text{-2-APA} \qquad (1)$$

In this earlier paper,[5] model explanation of the oscillatory transenantiomerization of the profens and amino acids belonging to

the class of 2-APAs was developed, based on the earlier devised model of the oscillatory processes known as Templator.[6,7] The novel model makes use of the two linked Templators, each one of them describing the different antimer.

In spite of the common structural features, the dynamics of the oscillatory structural conversion of the chiral 2-arylpropionic acids (2-APAs) – due to their differentiated molecular structure and, hence, to the different electron density distribution – must also differ. The present study was focused on checking if *L*-tyrosine (*L*-Tyr) can undergo the oscillatory transenantiomerization according to the following scheme:

$$L\text{-Tyr} \leftrightarrow \text{keto-enol tautomer} \leftrightarrow D\text{-Tyr} \qquad (2)$$

and, if so, how this process can be compared with that characteristic of *L*-α-phenylalanine.

EXPERIMENTAL

L-Tyrosine

The chemical structure of *L*-tyrosine is given in Figure 1. In our study, we used *L*-tyrosine purchased from Merck KGaA (Darmstadt, Germany; cat. # 1.08371.0025). In the thin-layer chromatographic experiments, we used solutions of *L*-tyrosine in ethanol-1M hydrochloric acid (7:3, v/v) and ethanol-1M sodium hydroxide (7:3, v/v), its concentration always equal to 1 mg mL^{-1} (i.e., ca. 5.5×10^{-3} M). The *L*-tyrosine solutions were stored for 8 days at 22 ± 2°C in order to trace the process of the oscillatory transenantiomerization. In certain time intervals the solution samples were analyzed by means of thin layer chromatography (TLC).

In the polarimetric experiment, we used the analogous two types of the *L*-tyrosine solutions, the amino acid concentration always equal to 50 mg mL^{-1} (i.e., ca. 2.76×10^{-1} M).

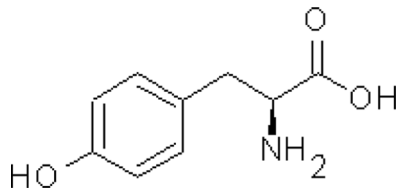


Figure 1. Chemical structure of *L*-tyrosine.

Polarimetric Measurements of the Specific Rotation $[\alpha]_D$

Measurements of the specific rotation ($[\alpha]_D$) of the *L*-tyrosine solutions in the ethanol-1M hydrochloric acid and ethanol-1M hydroxide sodium mixtures were carried out at $9 \pm 2°C$ and $22 \pm 2°C$ for 360 min (in 15-min intervals) without and with strirring (by ultrasonification of the measuring cell). Ultrasonification was performed with aid of the Model RK 255H Sonorex Super (Bandelin, Berlin, Germany) ultrasonification bath. Measurements of the specific rotation were carried out with use of a Polamat A polarimeter (manufactured by Carl Zeiss, Jena, Germany). The optical path length of the employed measuring cell was exactly 10 cm (=1 dm), and its volume was ca. 1 mL. Specific rotation $[\alpha]_D$ was calculated, using the following standard equation:

$$[\alpha]_D = 100\,\alpha/cd \tag{3}$$

where α is the measured rotation (in the angle degrees); D is the employed wavelength $\lambda = 589$ nm, which corresponds with the sodium D line; c is the concentration of a given compound in $g\ 100\,mL^{-1}$ solution; and d is the measured sample thickness in dm.

From the literature,[8] it is known that the specific rotation ($[\alpha]_D$) of *L*-tyrosine at 20°C equals ca. $-10.0° \div -12.3°$.

Commercial TLC Silica Gel Layers and their Pretreatment

TLC was performed on commercial glass plates (20 cm × 20 cm) precoated with 0.25 mm layers of silica gel 60 F_{254} (Merck, cat. #1.05715). Before use, the plates were washed by predevelopment with methanol-water, 9:1 (v/v), and then dried at ambient temperature ($22 \pm 2°C$) for 3 h.

The washed and dried plates were then twice impregnated by conventional dipping, lasting 2 s each. The first solution used for impregnation was a 2.39×10^{-2} M L^{-1} aqueous solution of $CuSO_4$ (analytical grade; manufactured by POCh, Gliwice, Poland). After the first impregnation, the plates were dried for 10 min at 105–110°C in a thermostatted chamber. The second solution used for impregnation was *L*-proline (*L*-Pro; Merck, cat. #1.07434.0010) in water – methanol (9:1, v/v), its concentration equal to 3.04×10^{-2} M. It is noteworthy that the molar ratio of the Cu^{2+} cations to the *L*-Pro molecules deposited on the chromatographic plates was equal to 1:2, representing in situ form the $[Cu(L\text{-Pro})_2]^{2+}$ complex. After the second impregnation, the plates were dried again at ambient temperature for 3 h, after which the impregnated adsorbent layers were ready for chromatography.

Mobile Phase and Development of Thin Layer Chromatograms

Development of the stored *L*-tyrosine samples was carried out at $22 \pm 2°C$ for a distance of 15 cm using the ternary mobile phase *n*-butanol (*n*-BuOH)-acetonitrile (ACN)-water (H_2O) (6:2:3, v/v). The anticipated mechanism of retention with each of the two tyrosine antipodes is given below:

$$[Cu(L\text{-Pro})_2]^{2+} + L\text{-Tyr} \leftrightarrow [Cu(L\text{-Pro})(L\text{-Tyr})]^{2+} + L\text{-Pro};\ K_1 \quad (4)$$

$$[Cu(L\text{-Pro})_2]^{2+} + D\text{-Tyr} \leftrightarrow [Cu(L\text{-Pro})(D\text{-Tyr})]^{2+} + L\text{-Pro};\ K_2 \quad (5)$$

Thus, the above mechanism of enantioseparation is known as the ligand exchange chromatography (or complexation chromatography). The TLC procedure employed in this study was adapted from that elaborated by Bhushan et al.[9] and first described[5] for the laboratory-coated chromatographic glass plates to use with commercially precoated plates.

Sample application to the plates was with the use of an autosampler (the AS 30 model autosampler manufactured by Desaga, Heidelberg, Germany). The tyrosine solutions were applied to the plate 1.5 cm above the lower edge of the plate in aliquots of 5 μL $spot^{-1}$. Nine samples in the equal distance of 2 cm from one another were applied per one plate, and then the chromatogram was developed in the one-dimensional ascending mode. After development, the plates were dried at ambient temperature for 3 h, and each development track was densitometrically scanned in 1-mm intervals at the width of 1 cm in the direction of development. Each experiment was carried out on two plates, thus the numerical results given in this paper originate from 18 individual development lanes.

Densitometric Assessment of the Thin Layer Chromatograms

Densitograms were acquired with a Desaga (Heidelberg, Germany) Model CD 60 densitometer equipped with Windows-compatible ProQuant software. Concentration profiles of the development lanes for α-phenylalanine were recorded in ultraviolet (UV) light from the deuterium lamp (in the reflectance mode) at 200 nm. The dimensions of the rectangular light beam were 2.0 mm × 0.1 mm. The maxima of the concentration profiles were used for calculation of retardation factor (R_F) values.

RESULTS AND DISCUSSION

Thin Layer Chromatography

The aim of the TLC experiment was to gather evidence on the occurence of the oscillatory transenantiomerization of *L*-tyrosine to its *D* antipode, with the *L*-tyrosine samples dissolved in the two different mixed solvents used in this study [i.e., ethanol – 1 M hydrochloric acid and ethanol – 1 M sodium hydroxide, 7:3 (v/v)], and then stored for eight days at $22 \pm 2°C$. Unlike our earlier studies on the oscillatory transenantiomerization of the selected profens and *L*-phenylalanine,[1,5] in this study we could not make use of the ethanol – water (7:3, v/v) mixture because *L*-tyrosine does not dissolve in this neutral solvent. In order to facilitate enantioseparation and to obtain neat Gaussian concentration profiles of the separated analytes, the *L*-tyrosine concentration in the fresh made solutions was purposely low (ca. 5.5×10^{-3} M).

In Figure 2, we show the oscillatory changes of the R_F values with the two different *L*-tyrosine solutions. These oscillatory changes are an important proof of structural conversion of *L*-tyrosine to its *D* antimer, as the investigated amino acid does not decompose under the applied storage conditions and the measuring experimental error of the R_F never surpasses ± 0.02 units. In our experiment, the maximum positions of the tyrosine band oscillate between the R_F values equal to 0.56 ± 0.02 and 0.35 ± 0.02), as shown in Figure 2.

From the results shown in Figure 2, it is seen that the average R_F values obtained for *L*-tyrosine stored in the ethanol-1 M hydrochloric acid mixture are lower than those for the same amino acid dissolved and stored in the ethanol-1 M sodium hydroxide mixture. This observation

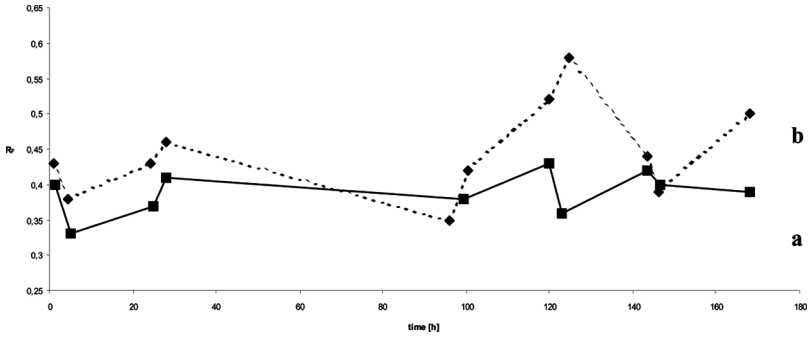


Figure 2. Dependence of retention, R_F, for *L*-tyrosine dissolved in (a) ethanol – 1 M hydrochloric acid, 7:3 (v/v), and (b) ethanol – 1 M sodium hydroxide, 7:3 (v/v) on sample storage time [$R_F = f(t)$] at $22 \pm 2°C$.

seems rather striking for the following reason. Attributing the higher R_F value to *L*-tyrosine and the lower one to its *D* antimer (which originates from the findings of Bhushan et al.[5]) might suggest that in the case of *L*-tyrosine the acidic medium better promotes its transenantiomerization to the *D* species than the basic one. Such a statement seems incorrect in view of basic knowledge on the reaction mechanisms in organic chemistry, of our earlier experience with the spontaneous *in vitro* chiral conversion of 2-APAs, and also in view of the polarimetric results reported in this study. There can be two alternative explanations to this question, which will remain unanswered in this study: (i) either attribution of the lower chromatographic peak to the *D* enantiomer and the higher one to its *L* antipode by Bhushan et al.[5] was erroneous, or (ii) in the employed chromatographic system *L*-tyrosine dissolved in hydrochloric acid undergoes rapid chiral conversion to *D*-tyrosine, and

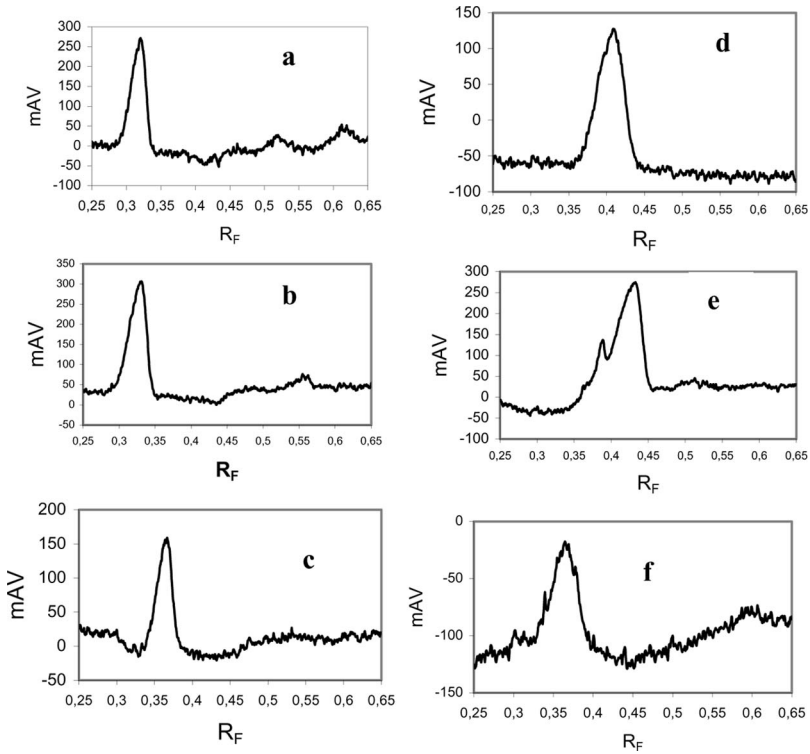


Figure 3. Sequence of densitometric concentration profiles for *L*-tyrosine solution in ethanol – 1 M hydrochloric acid, 7:3 (v/v) after (a) 1.3 h, (b) 5 h, (c) 25 h, (d) 28 h, (e) 120 h, and (f) 123 h storage time at 22 ± 2°C.

then its chirality is in a rather difficult to understand manner stabilized (which right now seems a less probable case).

An important proof of the oscillatory transenantiomerization of *L*-tyrosine effectively taking place in the reaction medium is not only the changing positions of the maximum of the analyte's concentration profile on the numerical scale of the R_F values, but also the changing shapes of these profiles. These changing maximum positions and shapes of the concentration profiles are presented in the form of a sequence of the individual 'movie pictures' for the sample dissolved in the ethanol-1M hydrochloric acid mixture [Figures 3(a)–(f)], and in the ethanol-1 M sodium hydroxide mixture [Figures 4(a)–(f)], and then stored for the period of eight days.

In the case of *L*-tyrosine dissolved in ethanol-hydrochloric acid, the obtained 'movie pictures' well show that the oscillation range of the respective R_F values is relatively narrow and the enantioseparation

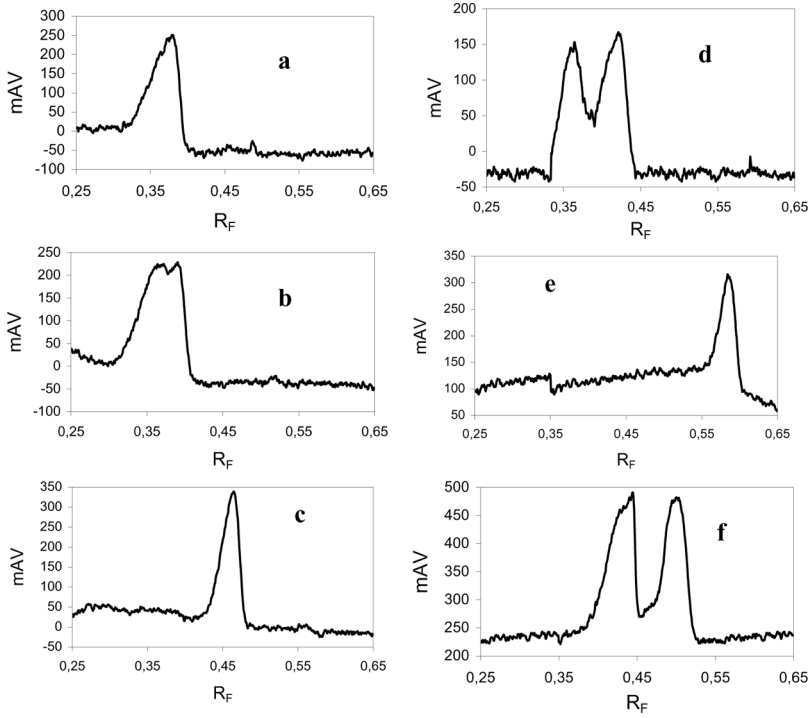


Figure 4. Sequence of densitometric concentration profiles for *L*-tyrosine solution in ethanol – 1 M sodium hydroxide, 7:3 (v/v) after (a) 1 h, (b) 4.6 h, (c) 28 h, (d) 100.5 h, (e) 124.5 h, and (f) 168 h storage time at 22 ± 2°C.

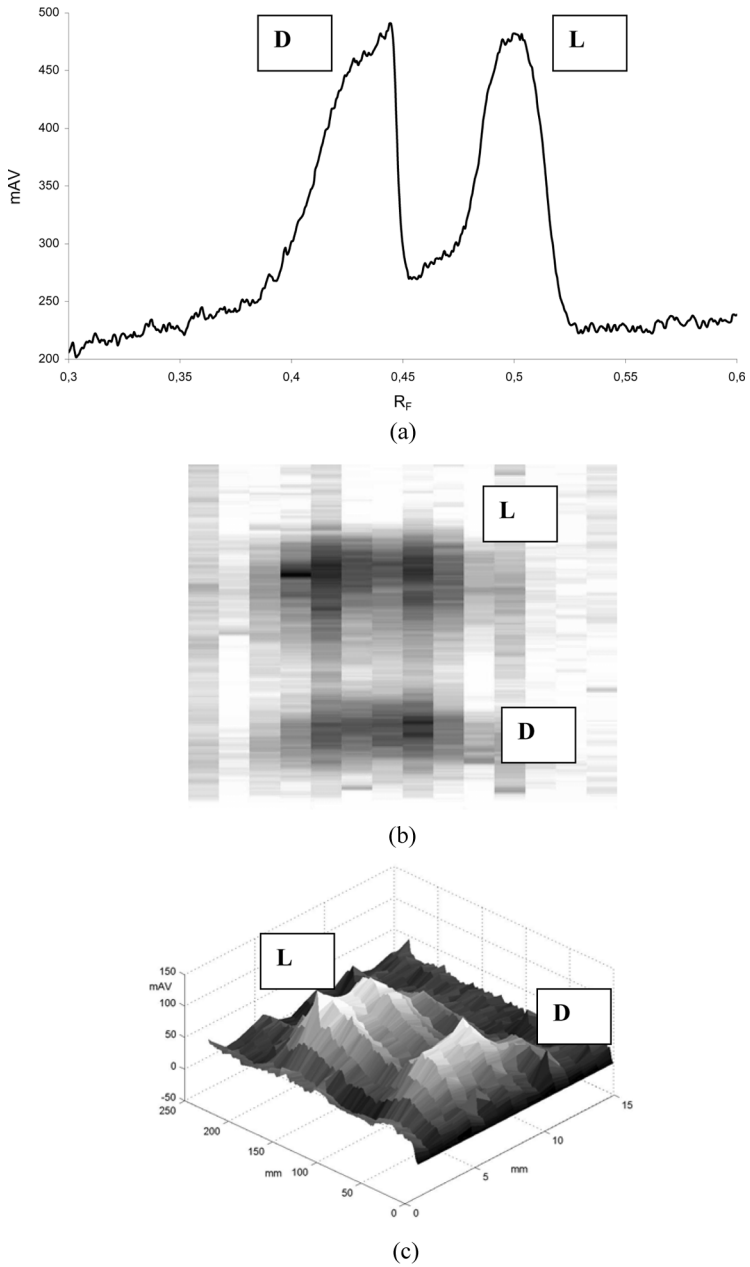


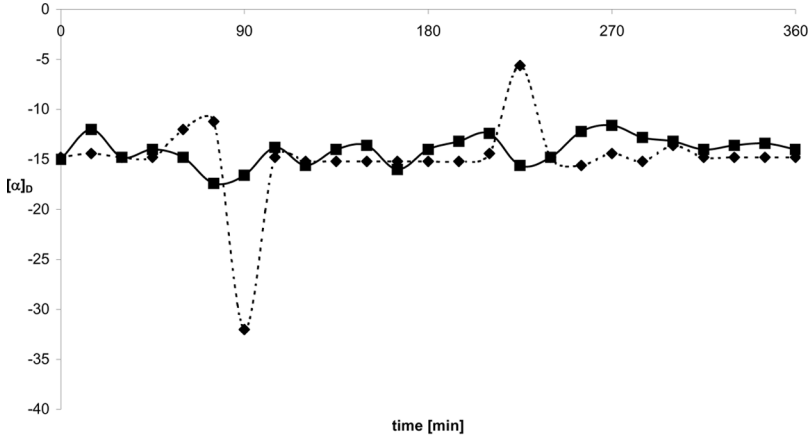
Figure 5. The 1D (densitogram) (a), 2D (b) and 3D (c) representation of the two enantioseparated chromatographic spots of *L*-and *D*-tyrosine after sample storage for 171 h in ethanol – 1 M sodium hydroxide solution (7:3, v/v) at 22 ± 2°C. The 2D and 3D pictures were drawn upon the densitometric scans of the separated pair of the *L*, *D*-tyrosine antimers taken in 1-mm intervals.

hardly occurs in the period of the 8 day experiment [Figures 3(a)–(f)]. With *L*-tyrosine dissolved in ethanol-sodium hydrochloride, the oscillation range is wider and the comparable quantitative proportions between the two antimers are apparently more frequent, which results in the more frequent cases of quite successful enantioseparations [Figures 4(a)–(f)]. In Figures 5(a)–(c), we present a typical example of enantioseparation of the tyrosine sample stored for a longer period of time (171 h) in the ethanol – 1 M sodium hydroxide solution.

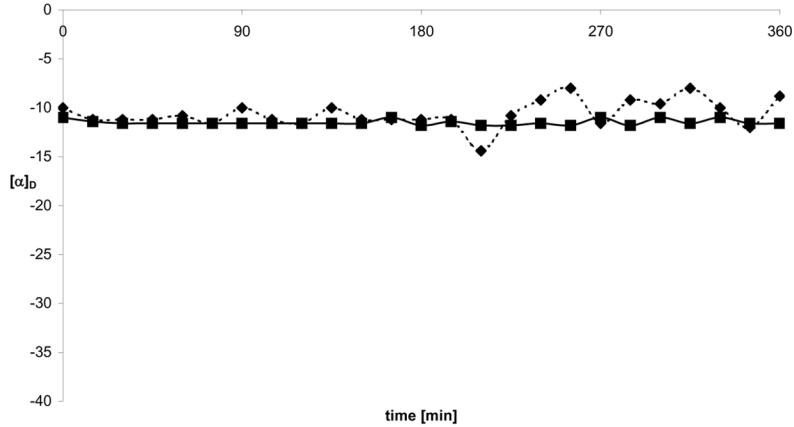
Polarimetry

Specific rotation of *L*-tyrosine in the ethanol-1 M sodium hydroxide and ethanol-1 M hydrochloric acid binary mixtures (with the amino acid concentration always equal to 2.76×10^{-1} M) was measured for 360 min by means of polarimetry at the two different working temperatures, 9 ± 2 and 22 ± 2°C. At each temperature, one sample was kept unstirred and the other was stirred by ultrasonification, in order to additionally investigate the effect of stirring.

At both working temperatures, without and with stirring, the specific rotation values for the *L*-tyrosine solutions were unstable, and they were undergoing the oscillatory changes. However, the amplitude of these oscillations at 22 ± 2°C was rather negligible and incomparably lower than at 9 ± 2°C. This effect can most probably be ascribed to the lower viscosity and/or self-organization of the amino acid molecules in the solutions kept at 22 ± 2°C and, hence, to the less pronounced density anisotropy of the investigated solutions, an important precondition of many chemical oscillatory processes. The results obtained at 9 ± 2°C are shown in Figures 6(a) and (b). From these plots the following conclusions can be drawn. Firstly, the amplitude of the oscillations with the stirred samples generally matches that of the unstirred samples. However, with *L*-tyrosine dissolved in the basic solvent, the amplitude of the oscillations both with the unstirred and ultrasonificated sample is much lower than that obtained in the analogous experiment with *L*-phenylalanine.[5] The observed difference is most probably due to the structural difference between these two amino acids, and more specifically due to the presence of an extra hydroxyl group in the molecule of *L*-tyrosine and in its absence in the molecule of *L*-phenylalanine. As *L*-tyrosine contains both the carboxyl and the hydroxyl moiety, it is considerably more apt to participate in intermolecular hydrogen bonding than *L*-phenylalanine. These hydrogen bonds can probably stabilize the electron distribution within the *L*-tyrosine molecules, thus weakening their tendency for chiral conversion.



(a)



(b)

Figure 6. Comparison of the oscillations of the specific rotation value ($[\alpha]_D$) for *L*-tyrosine dissolved and stored for 360 min at 9 ± 2°C in (a) ethanol – 1 M sodium hydroxide (7:3, v/v), and (b) ethanol – 1 M hydrochloric acid (7:3, v/v), without and with ultrasonification (solid line without ultrasonification; dashed line with ultrasonification).

Secondly, it is evident that the well perceptible amplitude of the oscillations is observed in the basic environment (Figure 6a), and a very modest one only in the acidic medium (Figure 6b). These results well coincide with our earlier findings[5,10] as to the catalytic role of a basic environment in the oscillatory steric conversion of chiral propionic acid derivatives (most probably running via the keto-enol tautomerism).

Model Explanation of the Oscillatory Transenantiomerization of *L*-Tyrosine

It seems understandable that the model explanation of the oscillatory transenantiomerization of *L*-α-phenylalanine presented earlier[5] is general enough to adequately explain the case of *L*-tyrosine and its oscillations also. In fact, that theoretical model can be applied to a wide number of the oscillatory chiral inversion cases happening with chiral low molecular weight carboxylic acids, amino acids, and profen drugs.

CONCLUSIONS

(i) The polarimetric results presented in this study are in good agreement with our concept of the keto-enol intermediary step in the oscillatory transenantiomerization of the chiral profens and amino acids from the group of 2-APAs. An assumption of this particular mechanism implies the catalytic effect of a basic environment and the hampering effect of an acidic environment on the investigated transenantiomerization process.

(ii) The thin layer chromatographic results presented in this study point to a more complicated pattern of the tranenantiomerization process in the chromatographic system than that in the bulk liquid phase, illustrated by the oscillations of the specific rotation ($[\alpha]_D$) values. An eloquent indication is that the oscillations of the R_F values are comparable for *L*-tyrosine stored both in the acidic and the basic medium. This is understandable in view of the chromatographic retention effect (i.e., the dynamic intermolecular interactions occurring between the analyte on the one hand and the remaining constituents of the chromatographic system on the other) that perceptibly adds to the overall mechanism of the investigated chiral conversion.

ACKNOWLEDGMENT

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REFERENCES

1. Sajewicz, M.; Piętka, R.; Pieniak, A.; Kowalska, T. Application of thin-layer chromatography (TLC) to investigating oscillatory instability of the selected profen enantiomers. Acta Chromatogr. **2005**, *15*, 131–149.

2. Sajewicz, M.; Piętka, R.; Pieniak, A.; Kowalska, T. Application of thin-layer chromatography (TLC) to investigate oscillatory instability of the selected profen enantiomers in dichloromethane. J. Chromatogr. Sci. **2005**, *43*, 542–548.
3. Sajewicz, M.; Piętka, R.; Pieniak, A.; Kowalska, T. Application of thin-layer chromatography to the investigation of oscillatory instability of selected profen enantiomers in physiological salt. J. Liq. Chromatogr. Rel. Technol. **2006**, *29*, 2059–2069.
4. Sajewicz, M.; Gontarska, M.; Kronenbach, D.; Wojtal, Ł.; Grygierczyk, G.; Kowalska, T. A study on the oscillatory in vitro transenantiomerization and enantioseparation of the flurbiprofen antimers by means of thin layer chromatography (TLC). Acta Chromatogr. **2007**, *18*, 226–237.
5. Sajewicz, M.; Gontarska, M.; Wojtal, Ł.; Kronenbach, D.; Epstein, I.R.; Kowalska, T. Experimental and model investigation of the oscillatory transenantiomerization of *L*-α-phenylalanine. J. Liq. Chromatogr. Rel. Technol. **2008**, *31*, 1986–2005.
6. Peacock-Lopez, E.; Radov, D.B.; Flesner, C.S. Mixed-mode oscillations in a self-replicating dimerization mechanism. Biophys. Chem. **1997**, *65*, 171–178.
7. Tsai, L.L.; Hutchinson, G.R.; Peacock-Lopez, E. Turing patterns in a self-replicating mechanism with a self-complementary template. J. Chem. Phys. **2000**, *113*, 2003–2006.
8. Virtual catalog of ScienceLab.com (http://sciencelab.com/)
9. Bhushan, R.; Reddy, G.P.; Joshi, S. TLC resolution of DL amino acids on impregnated silica gel plates. J. Planar Chromatogr.-Mod. TLC. **1994**, *7*, 126–128.
10. Sajewicz, M.; Piętka, R.; Drabik, G.; Kowalska, T. On the mechanism of oscillatory changes of theretardation factor (R_F) and the specific rotation $[\alpha]_D$ with selected solutions of *S*-(+)-naproxen. J. Liq. Chromatogr. Rel. Technol. **2006**, *29*, 2071–2082.

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